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Lidanpaidu prescription alleviates lipopolysaccharide-induced acute kidney injury by suppressing the NF-κB signaling pathway

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ABSTRACT

The Lidanpaidu Prescription (LDP), a hospital preparation, composed of Chinese classical preparations, has been reported to have antiendotoxin, anticoagulant and other effects. However, its therapeutic effect on lipopolysaccharide (LPS)-induced acute kidney injury (AKI) and the mechanisms remain unclear. Therefore, we administered LPD pretreatment at different doses to examine the protective effects and mechanisms in LPS-induced AKI in mice. The kidney injury induced by LPS was assessed by histological examination. ELISA was used to detect the levels of inflammatory cytokines. The mRNA expression of the inflammatory genes IKKβ and TNF-α in kidney tissues was assessed by RT-PCR. Finally, Western blot was performed to assess the NF-κB signaling pathway related proteins, and the nuclear translocation of NF-kB P65 was detected by immunofluorescence laser confocal microscopy. The findings suggested that LDP significantly improved at 48 h animal survival (66.7%), compared with the LPS group (26.7%), determined by a Kaplan-Meier analysis. LDP attenuated the kidney histopathological changes induced by LPS and decreased the inflammatory cytokine levels in serum and renal tissue. Moreover, LDP markedly inhibited the expression of inflammatory genes and suppressed the activation of relevant proteins in the nucleus. In summary, these findings suggest that LDP reduces LPS-induced AKI via a mechanism related to the suppression of the NF-κB signaling pathway.

1. Introduction

Sepsis is a systemic inflammatory response syndrome with a complex pathogenesis, mainly caused by invasive infection [1]. The main reasons for sepsis-induced death are an uncontrolled immune response, resulting in tissue and organ damage [2,3]. One of the most vulnerable target organs in endotoxin-induced sepsis is the kidney. More than 50% of patients in the ICU suffer from acute kidney injury (AKI) [4], and the mortality rate is as high as 30%–60% [5,6]. There are many therapeutic methods to investigate sepsis and its complications such as acute kidney injury [7], but there have been no significant decreases in its mortality rates.

Lipopolysaccharide (LPS) is found in the outer membrane of Gram-negative bacteria. In mice and other animal models, LPS induces AKI and leads to a strong inflammatory response via nuclear factor-kappa B (NF-κB) activation [8]. When AKI occurs, endotoxin induces the production of cytokines, resulting in a systemic “cytokines storm”, accompanied by activation of the NF-κB signaling pathway. NF-κB is an important transcription factor downstream of the endotoxin signaling transduction pathway. When inflammation occurs, I kappa B kinase beta (IKK beta) is activated first, then the activated inhibitor kappa B (IκB) kinase degrades the NF-κB inhibitory protein IκB and allows NF-κB to translocate into the nucleus [9,10]. Therefore, inhibiting the NF-κB signaling pathway might prevent AKI.

The Lidanpaidu prescription(LDP) is composed of capillary artemisia, gardenia, salvia and six other kinds of traditional Chinese medicine (TCM), combined with the Yinchenhao decoction and the Dachengqi decoction, according to TCM syndrome differentiation therapy theory. The Yinchenhao decoction and Dachengqi decoction are famous prescriptions in the “Treatise on Febrile Diseases” written by Zhang Zhongjing during the Han Dynasty. Research and clinical application have shown that the Yinchenhao decoction and the Dachengqi decoction both have good cholagogic and anti-inflammatory effects [11,12]. The antiendotoxin effect of LDP has also been demonstrated by in vivo and vitro experiments [13–15]. LDP has also been awarded a national patent (patent application number CN200710051818.6, patent publication number CN100589820C). In the clinical, it is mainly used for the treatment of infections, trauma and functional lesions caused by endotoxemia [13–16], and has a good curative effect on respiratory tract infections and urinary system infections, especially nephritis. However, its mechanism of action is not clear. In the present study, we investigated the protective effects and the mechanisms of action of LDP

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Table 1
The LDP prescription.

	TMC materials (pinyin)	Equivalent pharmaceutical name	Part used	Amount (g)
1	Yin chen	*ArtemisiacapillarisThunb*	seedling	30
2	Zhi zi	*Gardenia jasminoides Ellis*	fruit	15
3	Da huang	*Rheum palmatum L .*	radix and rhizome	15
4	Mang xiao	*Natrii Sulfas*	mineral	10
5	Gan cao	*Radix Glycyrrhizae*	radix and rhizome	5
6	Huang qi	*Radix Astragali seu Hedysari*	radix	15
7	Dan shen	*Radix Salviae Miltiorrhizae*	radix and rhizome	15
8	Jin yin hua	*Flos Lonicerae*	flower	15
9	Lian qiao	*Fructus Forsythiae*	fruit	10
Total				130

in AKI caused by LPS in mice.

2. Materials and methods

2.1. Chemicals and reagents

Lipopolysaccharide (LPS; 0111:B4) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). The FastQuant RT Kit and SuperReal PreMix Plus Kit were purchased from Tiangen Biotech Co. Ltd (Beijing, China). The Nuclear and Cytoplasmic Protein Extraction Kit, the BCA kit and BeyoECL Plus Kit were provided by Beyotime Co. Ltd. (Shanghai). TNF-α, IL-6, IL-10 ELISA kits were obtained from Bioswamp(Wuhan, China).

2.2. Preparation of LDP

The Lidanpaidu Prescription, supplied by Shiyan Taihe Hospital, was prepared according to the preparation method described by Zheng et al. [16]. All the herbal medicines (Table 1) in the preparation were shown to be endotoxin-free by Professor Keli Chen of Hubei University of Chinese Medicine, according to the Chinese Pharmacopoeia (2015 version). The component analysis of the extract has been provided by other authors [16–18].

2.3. Animals and treatments

In total, 108 mice (BABL/c mice, 6–8 weeks old) were purchased from the Hubei experimental animal research center (Wuhan, China). All procedures were approved by the Ethics Committee of Hubei University of Chinese Medicine. In the first group of studies, 60 BALB/c mice were randomly divided into four groups: control group (saline), LPS group (LPS 7 mg/kg), LPS + LDP (LDP 75 g/kg + LPS 7 mg/kg) group, LPS + dexamethasone hydrochloride [19] (5 mg/kg + LPS 7 mg/kg) group. All the mice were dosed by intragastric administration for seven days. One hour after the last intragastric administration, the LPS group, LPS + LDP group and dexamethasone group were injected intraperitoneally with LPS (7 mg/kg), and the control group was injected with normal saline in an equal volume. Then, the general condition and survival of the mice were monitored every 4 h up to 48 h and a survival curve was plotted. In the second round of studies, 48 mice were randomly divided into six groups (n = 8 each group): control group, LPS group (LPS 7 mg/kg), LPS + LDP (37.5, 75, and 150 g/kg + LPS 7 mg/kg) groups, and a LPS + dexamethasone hydrochloride (5 mg/kg + LPS 7 mg/kg) group. The method of administration was as described above. Six hours after the LPS injection, the mice were culled and the blood and kidney tissues were harvested.

2.4. Measurement of inflammatory cytokines

Retro-orbital blood samples were collected, then centrifuged at 4 °C for 20 min at 2500 rpm to collect the serum. The kidney tissues were ground in cold-PBS to obtain a homogenate. ELISA kits (Bioswamp) were used to measure TNF-α, IL-6 and IL-10 in serum and kidney homogenates, according to the manufacturer's protocol.

2.5. Histopathological examination

The kidney tissues were fixed in 10% formaldehyde, embedded in paraffin, then cut into 5 μm-thick slices followed by staining with hematoxylin and eosin (H&E). Histological changes were observed under a light microscope.

2.6. RT-PCR analysis

Total mRNAs was extracted from the kidney homogenates, and the purity and concentration of RNA were determined by a ultramicro UV detector. The mRNAs were reversely transcribed into first strand cDNA by the FastQuant RT Kit (Tiangen Biotech). Then semi-quantitative real-time PCR was conducted using the first strand cDNA and SuperReal PreMix Plus (SYBR Green) Kit (Tiangen Biotech) according to the manufacturer's instructions. Real-time qPCR was performed for 35 cycles in 20 μL reaction volumes using a Roche L480 Real-time PCR System. The upstream and downstream primer sequences were as described previously [20] as follows: for β-actin: sense primer: 5′-TTGT TACCAACTGGGACG-3′, antisense primer: 5′-GGCATAGAGGTCTTTA CGG-3′; for IKKβ: sense primer: 5′- AGGCGACAGGTGAACAGAT -3′, antisense primer: 5′- CTAAGAGCCGATGCGATG -3′; for TNF-α: sense primer: 5′- GGCAGGTCTACTTTGGAGTCATTGC -3′, antisense primer: 5′- ACATTCGAGGCTCCAGTGAATTCGG -3′. Cycling of IKKβ began at 95 °C for 15 min, then cycled 35 times: denatured at 95 °C for 20 s, annealed at 56 °C for 30 s and extended at 72 °C for 40 s. Cycling of

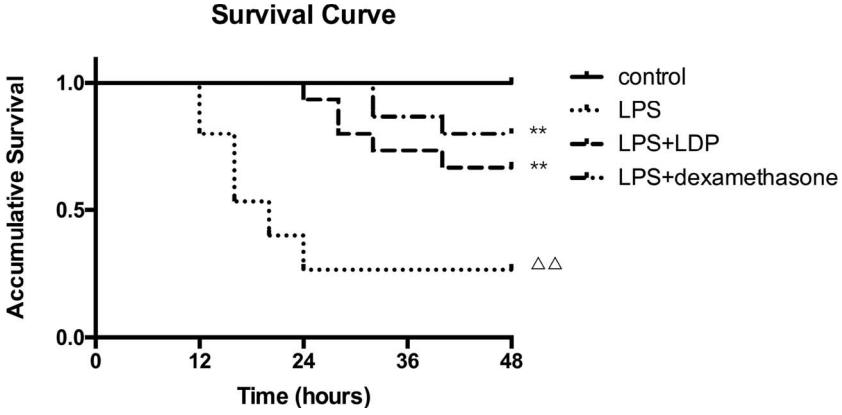

Fig. 1. Survival rate analysis. $^{\triangle\triangle}P < 0.01$ vs control group; $^{**}P < 0.01$ vs LPS group.